



PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Kovesdi et al.

Application No. 09/964,065

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Art Unit: 1632

Examiner: S.D. Priebe

For: REPLICATION-DEFICIENT ADENOVIRAL
VECTOR AND PLASMID WITH
ADENOVIRAL COMPONENT

PENDING CLAIMS AFTER AMENDMENTS

44. A plasmid comprising a reading frame ORF6 of an E4 region of an adenovirus genome under the control of a heterologous inducible promoter.

48. A defective recombinant adenovirus that (a) requires, for replication, complementation *in trans* of one or more essential gene functions of an E1 region and an E4 region of an adenovirus genome, and (b) comprises an adenoviral genome wherein all or part of the E1 region and the whole of the E4 region, and optionally all or part of an E3 region, is deleted from the adenoviral genome.

49. The defective recombinant adenovirus of claim 48, wherein part of the E1 region is deleted.

50. The defective recombinant adenovirus of claim 48, wherein all of the E1 region is deleted.

51. The defective recombinant adenovirus of claim 49, wherein all or part of the E3 region is deleted.

52. The defective recombinant adenovirus of claim 50, wherein all or part of the E3 region is deleted.

53. A system comprising:

(i) an adenoviral vector comprising an adenoviral genome having a deficiency in one or more essential gene functions of the E1 region of the adenoviral genome and a deficiency in one or more essential gene functions in either or both of the E2A region and the E4 region of the adenoviral genome, and optionally a deficiency in the E3 region of the adenoviral genome, and

(ii) a 293 cell or an A549 cell having a cellular genome that complements in *trans* for the deficiency in one or more essential gene functions of the E1 region of the adenoviral genome and the deficiency in one or more essential gene functions in either or both of the E2A region and the E4 region of the adenoviral genome,

wherein there is no overlap between the cellular genome and the adenoviral genome to mediate a recombination event between the cellular genome and the adenoviral genome.

54. The system of claim 53, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in all essential gene functions of the E1 region, and the cell has a cellular genome that complements in *trans* for the deficiency in all essential gene functions of the E1 region.

55. The system of claim 53, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in one or more essential gene functions of the E1 region of the adenoviral genome and a deficiency in one or more essential gene functions of the E4 region of the adenoviral genome and the cell has a cellular genome that complements in *trans* for the deficiency in one or more essential gene functions of the E1 region of the adenoviral genome and the deficiency in one or more essential gene functions of the E4 region of the adenoviral genome.

56. The system of claim 55, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in all essential gene functions of the E1 region, and the cell has a cellular genome that complements in *trans* for the deficiency in all essential gene functions of the E1 region.

57. The system of claim 55, wherein the cellular genome comprises at least open reading frame (ORF) 6 of the E4 region of the adenoviral genome.

58. The system of claim 57, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in all essential gene functions of the E1 region, and the cell has a cellular genome that complements in *trans* for the deficiency in all essential gene functions of the E1 region.

59. The system of claim 57, wherein the cellular genome comprises at least ORF6 and no other ORF of the E4 region of the adenoviral genome.

60. The system of claim 59, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in all essential gene functions of the E1 region, and the cell has a cellular genome that complements in *trans* for the deficiency in all essential gene functions of the E1 region.

61. The system of claim 53, wherein the cell is a 293 cell.

62. The system of claim 53, wherein the cell is an A549 cell.

63. A method of propagating an adenoviral vector, which method comprises
(a) providing an adenoviral vector comprising an adenoviral genome having a deficiency in one or more essential gene functions of the E1 region of the adenoviral genome and a deficiency in one or more essential gene functions in either or both of the E2A region and the E4 region of the adenoviral genome, and optionally a deficiency in the E3 region of the adenoviral genome,

(b) providing a 293 cell or an A549 cell comprising a cellular genome that complements in *trans* for the deficiency in one or more essential gene functions of the E1 region of the adenoviral genome and the deficiency in one or more essential gene functions in either or both of the E2A region and the E4 region of the adenoviral genome, wherein there is no overlap between the cellular genome and the adenoviral genome to mediate a recombination event between the cellular genome and the adenoviral genome, and

(c) propagating the adenoviral vector in the cell.

64. The method of claim 63, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in all essential gene functions of the E1 region, and the cell has a cellular genome that complements in *trans* for the deficiency in all essential gene functions of the E1 region.

65. The method of claim 63, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in one or more essential gene functions of the E1 region of the adenoviral genome and a deficiency in one or more essential gene functions of the E4 region of the adenoviral genome, and the cell has a cellular genome that complements in *trans* for the deficiency in one or more essential gene functions of the E1 region of the adenoviral genome and the deficiency in one or more essential gene functions in the E4 region of the adenoviral genome.

66. The method of claim 65, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in all essential gene functions of the E1 region, and the cell has a cellular genome that complements in *trans* for the deficiency in all essential gene functions of the E1 region.

67. The method of claim 65, wherein the cellular genome comprises at least open reading frame (ORF) 6 of the E4 region of the adenoviral genome.

68. The method of claim 67, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in all essential gene functions of the E1 region, and the cell has a cellular genome that complements in *trans* for the deficiency in all essential gene functions of the E1 region.

69. The method of claim 67, wherein the cellular genome comprises at least ORF6 and no other ORF of the E4 region of the adenoviral genome.

70. The method of claim 69, wherein the adenoviral vector comprises an adenoviral genome having a deficiency in all essential gene functions of the E1 region, and the cell has a cellular genome that complements in *trans* for the deficiency in all essential gene functions of the E1 region.

71. The method of claim 63, wherein the cell is a 293 cell.

72. The method of claim 63, wherein the cell is an A549 cell.